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Review

Collective unconscious: How gut microbes shape human behavior

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ABSTRACT

The human gut harbors a dynamic and complex microbial ecosystem, consisting of approximately 1 kg of bacteria in the average adult, approximately the weight of the human brain. The evolutionary formation of a complex gut microbiota in mammals has played an important role in enabling brain development and perhaps sophisticated social interaction. Genes within the human gut microbiota, termed the microbiome, significantly outnumber human genes in the body, and are capable of producing a myriad of neuroactive compounds. Gut microbes are part of the unconscious system regulating behavior. Recent investigations indicate that these microbes majorly impact on cognitive function and fundamental behavior patterns, such as social interaction and stress management. In the absence of microbes, underlying neurochemistry is profoundly altered. Studies of gut microbes may play an important role in advancing understanding of disorders of cognitive functioning and social interaction, such as autism.

Humans live in a symbiotic relationship with gut microbes: we provide them with a constant source of nutrition, while in return they help us in a variety of ways including enabling optimal brain development and subsequent functioning (Geurts et al. 2013; Chen et al., 2013). Many human genes are homologs of bacterial genes, mainly derived by descent, but some by gene transfer from bacteria (McFall-Ngai et al., 2013). The word commensal is derived from the Latin term “cum mensa”, which means “eating together”. It has been postulated that in the absence of bacteria humans would not have developed the current level of cognitive performance (Montiel-Castro et al., 2013) We are fundamentally dependent on a myriad of essential neurochemicals produced by microbes. For example, the brain's serotonergic system, which plays a key role in emotional activity, does not develop appropriately in the absence of microbes (Clarke et al., 2013). Gut microbes are part of the unconscious system regulating behavior. Of major importance for societal functioning is the human trait of sociability. We are social creatures with superior cognitive functioning, which enabled us to become the dominant species on the planet. In rodents, who are raised without any bacteria, one sees altered sociability with clear autistic-like patterns of behavior (Desbonnet et al., 2014). Within an evolutionary framework colonization with crowds of bacteria facilitated mammalian group living in social crowds (Lombardo, 2008; Troyer, 1984), and thus the capacity for environmental dominance. Here we will review the evidence in support of these assertions and the potential role of gut microbes in psychiatry. Unlike many recent reviews published in specialist journals, the current review is aimed at practicing psychiatrists.

1. Brain–gut–microbiota axis

The general scaffolding of the brain–gut–microbiota axis (BGM) includes the central nervous system (CNS), the neuroendocrine and neuroimmune systems, the sympathetic and parasympathetic arms of the autonomic nervous system (ANS), the enteric nervous system (ENS) and most importantly the intestinal microbiota (Mayer, 2011). These components interact to form a complex reflex network with afferent fibers that project to integrative CNS structures and efferent projections to the smooth muscle (Grenham et al., 2011). Through this bidirectional communication network, signals from the brain can influence the motor, sensory and secretory modalities of the gut and conversely, visceral messages from the gut can influence brain function (Grenham et al., 2011; Montiel-Castro et al., 2013). Less extensively studied, but increasingly appreciated, is the potential impact of the enteric microbiota on brain function (Khanna and Tosh, 2014). The gut microbiota

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consists of predominantly bacteria, but also contains archaea, protozoa, fungi and viruses, all of which have co-evolved with the human host. These microorganisms perform vital functions, which are essential for host health, including food processing, digestion of complex indigestible polysaccharides, synthesis of vitamins, and inhibition of pathogens (Cecchini et al., 2013; Ramakrishna, 2013).

2. Key communication routes

The bidirectional communication between gut microbes and the brain occurs via a number of routes (Fig. 1) (Dinan and Cryan, 2013). The vagus nerve (cranial nerve X) has both efferent and afferent divisions, and plays a fundamental role in enabling signals from brain to gut and *vice versa*. Activation of the vagus nerve has also been shown to have a marked anti-inflammatory capacity, protecting against microbial-induced sepsis, an impact mediated by the acetylcholine nicotinic receptor α7 subunit (Boeckxstaens, 2013). Many of the effects of the gut microbiota or potential probiotics (live bacteria with a health benefit) on brain function are dependent on vagal activation (Bercik et al., 2012). However, vagus-independent mechanisms are also at play in microbiota–brain interactions, as vagotomy fails to influence certain aspects of communication.

The immune system provides a further route of communication between gut microbes and the brain. Microbiota and probiotic agents have direct effects on the immune system. Indeed, the innate and adaptive arms collaborate to maintain homeostasis at the luminal surface of the intestinal host–microbial interface, which is crucial for maintaining health. The immune system also exerts a bidirectional communication with the CNS, making it a

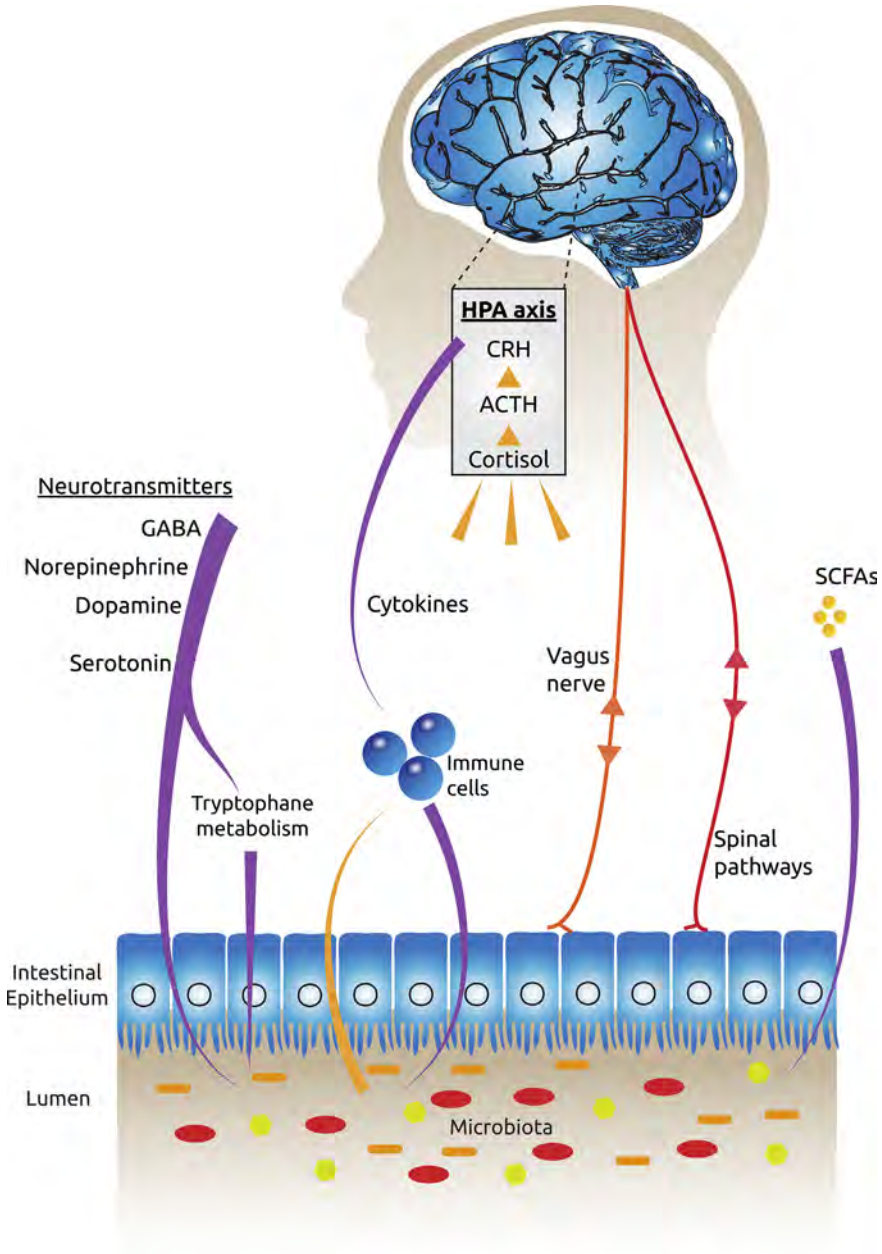

Fig. 1. The multiple bidirectional routes of communication between the brain and the gut microbiota. These routes include the vagus nerve, the hypothalamic-pituitary-adrenal axis (HPA), cytokines produced by the immune system, tryptophan metabolism and production of short chain fatty acids.

prime target for transducing the effects of bacteria on the CNS (Hueston and Deak, 2014). In addition, indirect effects of the gut microbiota and probiotics on the innate immune system can result in alterations in the circulating levels of pro- and anti-inflammatory cytokines that directly affect brain function, especially areas such as the hypothalamus, where interleukin (IL)-1 and IL-6 provide a potent release of corticotrophin releasing hormone (CRH) (Viswanathan et al., 2013). CRH is the dominant peptide regulator of the hypothalamic-pituitary-adrenal axis (HPA), which itself provides another bidirectional route of communication.

Tryptophan is an essential amino acid and is a precursor to many biologically active agents, including the neurotransmitter serotonin (5HT): most of the peripheral tryptophan is metabolized to kynurenine. The initial rate-limiting step in the kynurenine metabolic cascade is catalyzed by either indoleamine−2,3−dioxygenase or the largely hepatic-based tryptophan 2,3−dioxygenase (Clarke et al., 2012b). The activity of both enzymes can be induced by inflammatory mediators and by corticosteroids (Sainio, 1997; Zhou et al., 2012). There is some evidence to suggest that a probiotic bacterium, *Bifidobacterium infantis*, can alter concentrations of kynurenine (Clarke et al., 2012a).

Gut bacteria contribute to host metabolism, for example by production of metabolites such as bile acids, choline and short-chain fatty acids (SCFAs) that are essential for host health. Indeed, complex carbohydrates such as dietary fiber can be digested and subsequently fermented in the colon by gut microorganisms into SCFAs such as n−butyrate, acetate and propionate, which are known to have neuroactive properties (Russell et al., 2013). Certainly, these SCFAs can enter the blood and act in brain regions through two discrete 7-transmembrane G protein coupled receptors (Tan et al., 2014), free fatty acid receptor 2 (FFA2) and FFA3.

Bacteria also have the capacity to generate many neurotransmitters and neuromodulators. For example, certain *Lactobacillus* and *Bifidobacterium* species produce gamma-aminobutyric acid (GABA); *Escherichia*, *Bacillus* and *Saccharomyces* spp. produce norepinephrine (NE); *Candida*, *Streptococcus*, *Escherichia* and *Enterococcus* spp. produce 5HT; *Bacillus* produces dopamine (DA); and *Lactobacillus* produces acetylcholine (Lyte, 2011, 2013; Wikoff et al., 2009). Some probiotics can modulate the concentrations of opioid and cannabinoid receptors in the gut epithelium (Rousseaux et al., 2007). However, how this local effect occurs or translates to the anti-nociceptive effects seen in animal models of visceral pain is currently unclear. It is conceivable that secreted neurotransmitters of microorganisms in the intestinal lumen may induce epithelial cells to release molecules that in turn modulate neural signaling within the ENS, or act directly on primary afferent axons (Mcvey Neufeld et al., 2013).

The outer extracellular polysaccharide coating of probiotic bacteria is largely responsible for many of their health-promoting effects (Claes et al., 2010). Indeed, the extracellular polysaccharide of the probiotic *Bifidobacterium breve* UCC2003 protects the bacteria from acid and bile in the gut and shields the bacteria from the host immune response. Indeed, as with neuroactive metabolites, cell wall components of microorganisms in the intestinal lumen or attached to epithelial cells are poised to induce epithelial cells to release molecules that in turn modulate neural signaling or that act directly on primary afferent axons.

3. Colonization of the gut

There are approximately 150−200 common and approximately 1000 less frequent bacterial species in the gut. The genes encoded by the human gut microbiota, collectively referred to as the microbiome, are over 100-fold more abundant than the genes of the human genome (Hamady and Knight, 2009). The colon harbors the greatest numbers of microorganisms in the human gut, with the vast majority of these indigenous microbes being strict anaerobes (Eckburg et al., 2005). The total weight of microbes in the adult human intestine is approximately the same weight as the human brain.

The composition and function of the intestinal microbiota has been the subject of intense scrutiny (Box 1), first using culture-dependant microbiological techniques (Grenham et al., 2011) and more recently, using culture-independent 16S rRNA gene sequence-based techniques, which allow greater insight into the microbial composition and diversity of this complex (Armougom and Raoult, 2008; Qin et al., 2010). While advances in metagenomic technologies have revealed the composition of the human gut microbiota from early infancy (Palmer et al., 2007) through to elderly (Claesson et al., 2012), far less is known about the physiological impact this microbiota has on host health, including that of the brain. Indeed, understanding the influence of the gut microbiota on host health has been described as one of the most exciting areas in all medicine (Shanahan, 2012).

At birth the human brain is highly under-developed and the gut is generally regarded as entirely sterile. However, it is worth noting that there is an increasing body of evidence challenging the sterile-womb paradigm (Funkhouser and Bordenstein, 2013) and that transmission of certain microbes already occurs *in utero*. Nonetheless, passage through the birth canal exposes the baby to the mother's microbiota. Initial colonization is dictated by the mother's microbes and the hospital environment. This colonization plays a fundamental role in brain development in the early post-natal weeks. The subsequent microbial composition of the neonatal gut is influenced by a number of factors including antibiotic use, diet, mode of delivery, environmental factors, and the maternal microbiota (Koenig et al., 2011; Marques et al., 2010; Dominguez-Bello et al., 2010). For example, the microbiota of formula-fed infants has been reported to be more diverse than breastfed infants, containing higher proportions of *Bacteroides*, *Clostridium*, and *Enterobacteriaceae*. Furthermore, vaginally delivered infants are colonized by the fecal and vaginal bacteria of the mother, whereas infants delivered by caesarean section (CS) are colonized by other bacteria from environmental sources including health-care workers, air, medical equipment and other newborns (Faa et al., 2013). Efforts to replicate the intestinal microbiota of breastfed infants via formula-feeding have led to the incorporation of prebiotics (food ingredients that selectively stimulate the growth and/or activity of one or more bacterial species in the colon and thereby beneficially affect the host) in infant formulae. It has been reported that a combination of galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS), at ratios similar to the oligosaccharide composition of human breast milk, can stimulate the growth of bifidobacteria and influence species distribution in the gut microbiota, and SCFA production levels to mimic those of breastfed infants (Park et al., 2013). Recent rodent studies have also shown that prebiotic supplementation with FOS and GOS can increase the expression of neurotransmitters and neuromodulators in the hippocampus, a key brain area involved in learning and memory (Savignac et al., 2013; De Vadder et al., 2014).

4. Gut microbiota components

The intestinal microbiota of newborn infants is characterized by low diversity and a relative dominance of the phyla Proteobacteria and Actinobacteria in the early period following birth. As time after birth increases, the microbiota becomes more diverse with the emergence and dominance of Firmicutes and Bacteroidetes. Full-term, vaginally delivered, breastfed, non-antibiotic treated infants are optimal for the development of the neonatal microbiota (Alex

et al., 2013). In these infants, facultative anaerobes such as enterobacteria, staphylococci and streptococci are the earliest to colonize, taking advantage of the redox potential and available oxygen of the newborn gut. These earliest colonizers consume the oxygen in the gut, thereby creating an anaerobic environment which allows the proliferation of the strict anaerobes, *Clostridium*, *Bacteroides* and bifidobacteria, with bifidobacteria becoming dominant and outnumbering all other bacterial groups and species within the first weeks of life. The neonatal microbiota is highly dynamic and is characterized by instability and low diversity. By the end of the first year of life, infants possess a microbial profile distinct for each infant, converging towards the characteristic microbiota of an adult and by 2.5 years of age the microbiota fully resembles that of an adult in terms of composition (Lobo et al., 2014).

The adult microbiota has been reported to be relatively stable over time in addition to being more complex than that of the neonate (Hamady and Knight, 2009). There are large interpersonal differences in the microbiota of healthy adults, even between identical twins. However, a shared core gut microbiome exists, exerting a common functionality within the host. The gut microbiota of adults is dominated primarily by members of the Bacteroidetes and Firmicutes phyla and more recently the adult microbiome was classified into three different 'enterotypes'. These 'enterotypes' are dominated by *Prevotella*, *Ruminococcus* and *Bacteroides*, respectively, and appear to be independent of sex, age, nationality and body mass index (BMI) (Arumugam et al., 2011).

5. Microbes and neurotransmission

Which of the 4–5 million non-redundant bacterial genes, which have been sequenced from the human gut, are fundamental for brain development? At this point we do not know. It is tempting to speculate that those microbes, which produce and secrete neuromodulators or their precursors, have an especially important role to play in neurodevelopment. GABA, which is the main inhibitory neurotransmitter in the human brain, is produced by many lactobacilli, and for most babies born *per vaginum* are the first bacteria to which they are exposed. As mentioned above other essential neurotransmitters such as 5HT, NE, DA are also produced by microbes.

There are a variety of techniques used for studying the impact of the gut microbiota on the brain: these techniques include germ-free (GF) studies in mice, antibiotic and probiotic studies, infection studies and fecal transplantation (Cryan and Dinan, 2012; Foster and McVey Neufeld, 2013). Matsumoto et al. (2013) assessed the cerebral metabolome of GF mice and found 196 metabolites of which 23 were at least 1.6 fold higher in GF than in ex-GF mice, while for 15 metabolites the reverse was true. Metabolites involved in glycolytic pathways were significantly higher in GF than in ex-GF mice. Concentrations of DA were two-fold higher in GF than in ex-GF mice. In the cerebrum of ex-GF mice the concentration of tryptophan, the precursor of 5HT was enhanced but they failed to find differences in 5HT levels. Levels of GABA were similar in the brains of GF and ex-GF mice but plasma concentrations differed. This data is consistent with the view that GABA produced by gut bacteria influences the brain via the vagus nerve and not by directly acting within the brain.

A recent study (Clarke et al., 2013) demonstrated that the consequences of growing up GF extends to a clear increase in the hippocampus in both 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), its main metabolite, over the normal levels of these neurochemicals. Despite the increased 5-HT concentration observed, there was no altered expression of the *Tph2* gene, the key CNS isoform of the enzyme responsible for the synthesis of 5-HT from tryptophan. No alteration in gene expression level was found for either the SERT (serotonin transporter) gene or the range of serotonergic receptor genes evaluated (5-HT_{1A}, 5-HT_{6} and 5-HT_{2C}). The alterations observed are sex-specific, occurring only in males, in contrast with the immunological and neuroendocrine effects, which are evident in both sexes. Concentrations of tryptophan, the precursor of serotonin, are increased in the plasma of male GF animals, suggesting a humoral route through which the microbiota can influence CNS serotonergic neurotransmission. Interestingly, colonization of the GF animals post-weaning is insufficient to reverse the CNS neurochemical consequences in adulthood of an absent microbiota in early life despite the peripheral availability of tryptophan being restored to baseline values. In addition, reduced anxiety in GF animals is also normalized following restoration of the intestinal microbiota. An earlier study by Desbonnet et al. (2008) reported increases in tryptophan levels in rats treated with the probiotic *Bifidobacterium infantis* 35624. This probiotic has been shown to have antidepressant action in preclinical models of depression and may thus constitute a psychobiotic with a mental health benefit (Dinan et al., 2013). Taken together, cumulating evidence points to key functions of microbial genes in neuronal function, supplying the developing as well as the mature brain with numerous neuroactive compounds, which have impact on health and disease, and may lie at the heart of behavioral disturbances when absent (Fig. 2).

6. BDNF and microbes

BDNF plays a pivotal role in supporting the survival of existing neurons, and encourages the growth and differentiation of new neurons and new synapse formations in the brain (Nicodemus et al., 2014). It is especially active in areas which play a key role in learning, memory, and higher cognitive activity, most notably the hippocampus and cortex. It is now a decade since Sudo and colleagues first reported that GF mice have decreased levels of BDNF protein in the hippocampus and cortex (Sudo et al., 2004). A similar finding was reported by Clarke et al. (2013), but the reduction was only seen in male and not in female mice. Neufeld et al. (2011) reported a downregulation of BDNF mRNA in the dentate region of the hippocampus in GF mice. In rats the probiotic *B. breve* 6330 increased BDNF total variants, and decreased BDNF splice variant IV in the hippocampus (O'sullivan et al., 2011). How might the microbiota influence BDNF levels? Some data indicate the involvement of short chain fatty acids. Butyrate, a histone deacetylase inhibitor, has been shown to influence BDNF expression in the hippocampus (Martins-De-Souza, 2010). In a similar vein Bercik and colleagues (Bercik et al., 2011) showed that administration of oral antimicrobials to mice increased hippocampal expression of BDNF; however, these changes were independent of inflammatory activity, changes in levels of gastrointestinal neurotransmitters, and vagal or sympathetic integrity. This group also demonstrated that the probiotic *Bifidobacterium longum* normalized the changes in behavior and BDNF mRNA induced by an intestinal parasite.

7. Microbes and cognition

Post-natal gut microbial colonization occurs in parallel with cognitive development. There is increasing evidence to support the view that the evolving cognitive activity is critically dependent on the microbiota and its metabolic activity. Gareau et al. (2011) used a novel object recognition test and exploration of a T-maze to examine dorsal hippocampal function in GF mice. The GF animals displayed an absence of non-spatial and working memory accompanied by decreases in hippocampal brain derived neurotrophic factor (BDNF) staining. The data indicate that compared with colonized animals, GF mice display significant cognitive deficits.

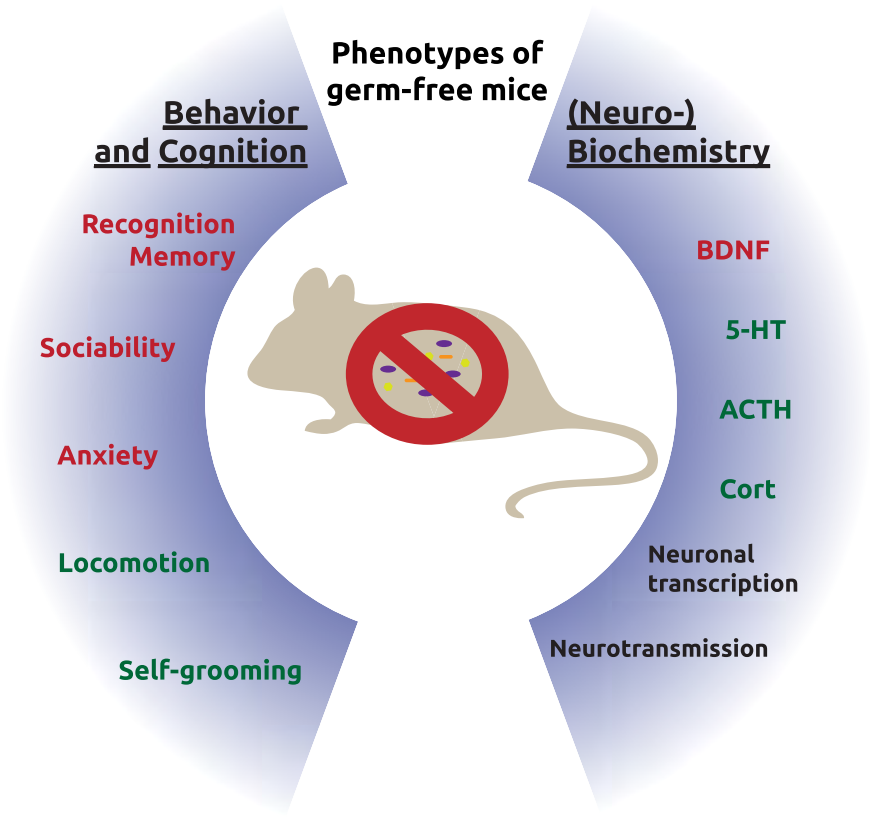

Fig. 2. Some of the major deficits in cognition, behavior and neurochemistry observed in germ-free mice. Abbreviation: Cort = cortisosterone.

The authors conclude that the presence of microbes is crucial for the development of hippocampus-dependent memory. In another study, Wall et al. (2012) demonstrated that ingestion of *B. breve* NCIMB702258, but not *B. breve* 6330, significantly impacted brain fatty acid composition in mice, elevating arachidonic acid (AA) and docosahexaenoic acid (DHA) concentrations, with potential clinical implications as these fatty acids play important roles in cognitive processes such as memory and learning.

Disorders of cognitive function such as Alzheimer's disease and multi-infarct dementia have a high prevalence after the age of 65 years, with at least 40% of individuals at 85 years of age showing some evidence of cognitive impairment (Hampel et al., 2011). A causal association between gut microbes and cognitive decline in the elderly has not yet been explored. Differences in the microbiota of elderly subjects have been reported, with frail elderly and those with cognitive dysfunction having the lowest diversity in gut microbial composition (Claesson et al., 2011, 2012). The ElderMet consortium was established with the aim of characterizing the gut microbiota of elderly (>65 years of age) Irish subjects. Recently, the consortium demonstrated that Bacteroidetes and Firmicutes are the dominant phyla in the distal gut of the elderly population, contributing to 97% of the assigned sequences (Claesson et al., 2011, 2012). However, when the diversity of each individual was examined, huge variations at phylum level were evident. For example, Firmicutes varied from 8% to 80%, whereas the phylum Bacteroidetes varied from 14% to 92%. These studies reported that 53% of the core microbiota in elderly subjects comprised of Bacteroidetes, compared with 8%–27% in younger adults.

It is therefore tempting to speculate that a changing microbiota in the elderly also acts as a modulator of inflammatory processes in the brain, which underlie many age-associated neurological diseases, including dementia and especially Alzheimer's disease. Diabetes mellitus is a well-established risk factor for dementia, and a recently completed trial of a synbiotic (*Lactobacillus acidophilus* plus prebiotic), which used a double-blind randomized design, showed that consumption of the synbiotic food for 6 weeks had significant positive effects on serum insulin, C reactive protein and uric acid (Asemi et al., 2014).

8. Microbes and social interaction

Mammals and especially humans are highly social beings. Indeed, evolution may have favored the development of social networks, and thereby increased capacity of our brain, to enhance the transfer of beneficial symbiotic microbes (Montiel-Castro et al., 2013). Understanding the development of neuronal circuitries underlying the social brain across the lifespan is one of the major efforts in neuroscience today. That microbes may play a role in the development of sociability has been proposed (Montiel-Castro et al., 2013; Troyer, 1984; Lombardo, 2008) in the context of evolutionary-based theories of the benefits of mutualism in social survival. In the following, we review the latest evidence for the hypothesis that microbes shape human social behavior.

Autism spectrum disorders (ASD) are neurodevelopmental disorders defined by deficits in social interaction and communication and the presence of limited, repetitive stereotyped interests and behaviors (Autism, 2012). An increasing number of pre-clinical studies are also supporting a link between ASDs and the microbiota-gut-brain axis (Fig. 3). deTheije et al. (2013) used a murine model of ASD to investigate the relation between the gut

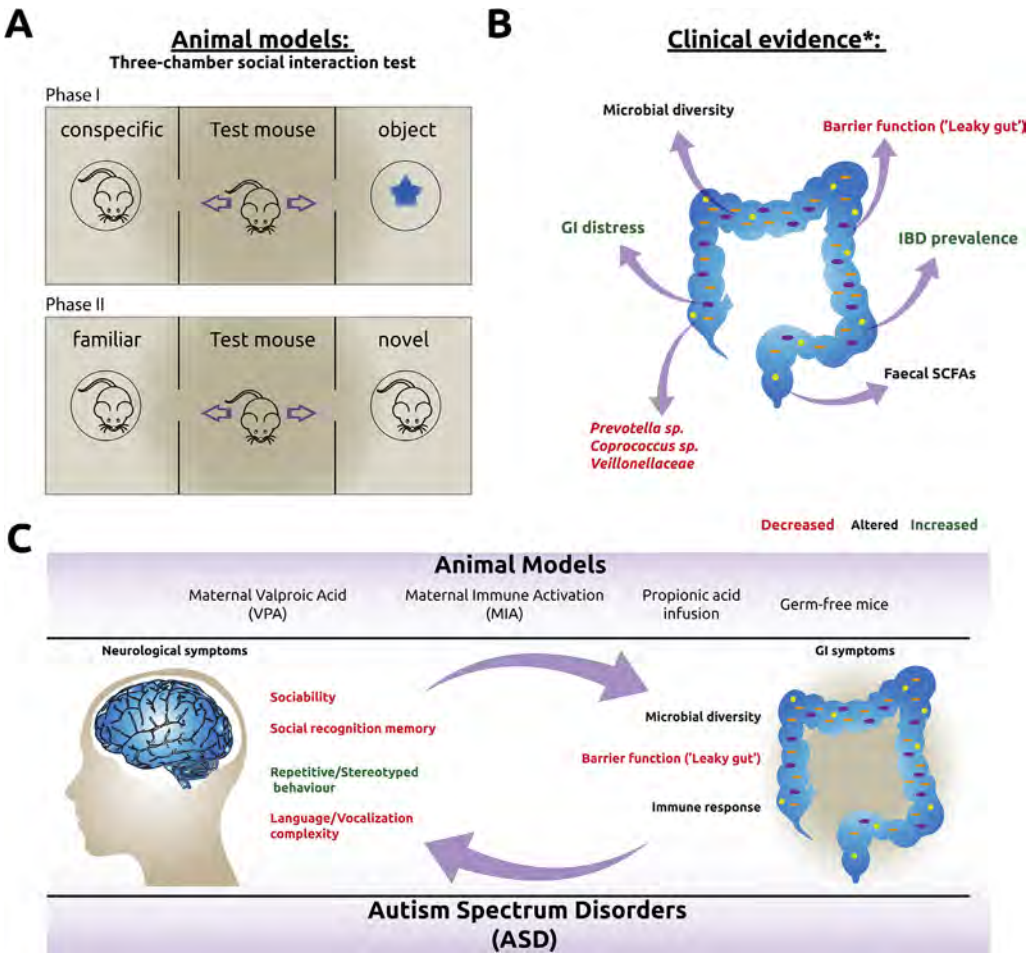

Fig. 3. How animal models can be used to study the etiology and treatment of autism. **(A)** The three-chamber social interaction test is commonly used to investigate social behavior such as sociability (Phase I) and social recognition memory (Phase II) in rodents. **(B)** Common gastrointestinal (GI) symptoms accompanying ASD diagnosis. *: the clinical picture is not homogenous and most likely incomplete. Several symptoms may be observed only in a subset of cases. **(C)** Phenotype observed in current animal models of ASD. Abbreviations: IBD, inflammatory bowel disease.

microbiota and autistic behavior. Using next generation sequencing technology, the composition of the gut microbiota was investigated in mice *in utero* exposed to valproic acid (VPA), an anticonvulsant and epigenetic modulator. Furthermore, the levels of SCFAs and lactic acid in feces were determined. The data demonstrate a transgenerational impact of *in utero* VPA exposure on gut microbiota in the offspring. Such VPA exposure affected the main phyla of Bacteroidetes and Firmicutes and the order of Desulfovibrionales. In addition, alterations in *Alistipes*, *Enterorhabdus*, *Mollicutes* and *Erysipelotrichalis* were especially associated with male VPA-exposed offspring. The microbial differences of VPA *in utero*-exposed males deviated from those observed in females and was (i) positively associated with increased levels of butyrate and (ii) inversely associated with intestinal levels of serotonin and social behavior scores.

The maternal immune activation mouse model is known to display features of autism. Hsiao et al. (2013) showed that maternal immune activation offspring display an altered serum metabolomic profile. They used oral treatment of such offspring with the human commensal *Bacteroides fragilis (B. fragilis)*. Treatment corrected gut permeability, altered microbial composition, and ameliorated defects in communicative, stereotypic, anxiety-like and sensorimotor behaviors. Treating naive mice with a metabolite that is increased by maternal immune activation and normalized by *B. fragilis* resulted in behavioral abnormalities, supporting the view that gut bacterial effects on the host metabolome impact behavior. Taken together, these findings suggest a gut–microbiome–brain connection in a mouse model of autism and identify a potential probiotic therapy.

Desbonnet et al. (2014) examined social behavior in GF mice using the three chamber sociability test. In the first study a GF mouse was placed in the middle chamber with a second mouse in the first chamber and an object in the third. A conventionally colonized mouse tends to spend more time with a second mouse than with an object, while the GF mouse spends far less time with another mouse and a greater amount of time with the object. In a second experiment, a GF mouse or a conventionally colonized mouse is placed in the middle chamber with a familiar mouse in the first chamber and an unfamiliar mouse in the third chamber. The conventionally colonized mouse spends more time with the unfamiliar mouse while the GF mouse shows no preference for either the familiar or unfamiliar mouse. The overall pattern of behavior in GF mice is therefore highly parallel to symptoms observed in autistic patients. Yet, there is a capacity for reversal if the animal is colonized at an early stage.

Altered faecal concentrations of SCFAs have also been reported in ASD (Allen, 1997). Of note is the fact that administration of propionic acid, an SCFA, to animals via the intracerebroventricular route results in some autistic-like behaviors (Couillin et al., 1994), albeit it at high doses that might not reflect the clinically observed alterations.

Changes in the microbiota of autistic patients have been reported (Cao et al., 2013), but as Gonzalez et al. (Collins et al., 2012) point out, studies of the gut microbiota in children with autism have been very limited, usually with small sample sizes, shallow sequencing of bacterial 16S rRNA gene amplicons, and without concomitant analyses of (i) the microbiome gene content (by shotgun sequencing of total faecal community DNA), (ii) microbiome gene expression, or (iii) microbial metabolism. Thus, the studies undertaken so far do not yet produce a homogenous or complete picture and clearly more systematic studies are needed. However, several studies repeatedly report a high co-morbidity of ASDs with gastrointestinal symptoms.

In conclusion, these data point to a critical role of microbial cues that affect brain development and especially impacts those neuronal circuitries that underlie social behavior in mammals. However, while there have undoubtedly been advances in animal models of ASD, some results from the various models are difficult to reconcile (Tania et al., 2014).

9. Microbiota and stress

The Yerkes-Dodson law states that stress can have a positive impact to a certain pivot point, beyond which the influence is deleterious (Bregman and Mcallister, 1983). The position of the pivot point is determined by multiple factors including the gut microbiota. Seminal studies by Sudo and colleagues (Sudo et al., 2004) provide insight into the role of the intestinal microbiota in the development of the core stress axis, the HPA. In GF mice, a mild restraint stress induces an exaggerated release of corticosterone and adrenocorticotropic hormone (ACTH) compared to the specific pathogen free (SPF) controls. The aberrant stress response in GF mice is reversed by monoassociation with *B. infantis* in a time dependent manner. This study clearly demonstrated that the microbial presence in the gut is critical to the development of an appropriate stress response later in life and also that there is a narrow window in early life where colonization must occur to ensure normal development of the HPA axis.

Early maternal separation produces long-term alterations in behavior, the HPA and the gut microbiota. Using such a model in rats, Desbonnet et al. (2008) found that treatment with the probiotic *B. infantis* normalized stress-related behavior but did not reduce corticosterone. Using a similar model Gareau et al. (2011) found that feeding a *Lactobacillus spp*. reduced corticosterone levels. Surprisingly, data from studies in rodents suggest that an anxious or a non-anxious phenotype can be induced with a microbiota transplant from an animal with a similar phenotype (Collins et al., 2013).

In a ground breaking functional magnetic resonance imaging (fMRI) brain scan study Tillisch et al. (2013) showed that ingestion of a probiotic cocktail altered information processing of emotional material. In another human intervention study, healthy volunteers were given *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 in combination or placebo in a double-blind, randomized parallel group study for 30 days. Twenty-four hour urinary free cortisol (UFC) output was reduced with probiotic treatment (Messaoudi et al., 2011). These studies provide important evidence that psychobiotics can alter mental processes and reduce stress responses. Resilience to environmental stress seems to be heavily influenced by microbial composition. This effect is most likely dependent on alterations of the HPA axis, and can be positively influenced by certain probiotic bacterial species. In support of this suggestion is the recent finding that prebiotic treatment, which is known to increase probiotic levels in the gut, alters the cortisol awaking response and emotional reaction in healthy subjects (Schmidt et al., 2014). Since stress beyond the pivot point is becoming an increasing burden for our societies, we should now aim to translate these findings into safe supplements and dietary recommendations.

10. Concluding remarks

During the course of evolution, multicellular life emerged from unicellular life forms. The latter remain the dominant life form on the planet and often exist in a symbiotic or parasitic relationship with multicellular life. The nature of such relationships can have a major influence on the life cycle of plants and animals. Here, we propose that the development of a complex gut microbiota in mammals has played an important role in enabling brain development, especially in terms of cognitive function and fundamental behavior patterns, such as facilitating social interaction and effectively dealing with environmental stressors. Thus, future studies of how gut microbes contribute to the function of their host on all levels will play an important role in advancing understanding of disorders of cognitive functioning as well as disorders of social interaction such as autism and a variety of stress-related conditions. Within a relatively short period of time, results of clinical trials of probiotics (psychobiotics) and prebiotics in the treatment of common psychiatric disorders will be available.

Conflict of interest

The Alimentary Pharmabiotic Centre has conducted studies in collaboration with several companies including GSK, Pfizer, Cremo, Suntory, Wyeth and Mead Johnson. TGD has until recently been on the Board of Alimentary Health. The authors have spoken at meetings sponsored by food and pharmaceutical companies.

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